81. A process according to claim 67 wherein a gene product is produced from the systemic transcription of the second nucleic acid sequence, the gene product being selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, EPO, G-CSF, GM-CSF, hPG-CSF, M-CSF, Factor VIII, Factor IX, tPA, hGH, receptors, receptor antagonists, antibodies, neuro-polypeptides, melanin, insulin and vaccines.

82. A process according to claim 67 wherein a gene product is produced from the systemic transcription of the second nucleic acid sequence, the gene product being a biologically inactive polypeptide or protein resulting from anti-sense RNA expression.
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REMARKS

The present Amendment is in response to the Examiner's telephone restriction requirement of November 19, 1993. Applicants presently cancel claims 1-42 and introduce new claims 45-82 which serve to more clearly define that which Applicants consider to be their invention. Submitted herewith in support of the present Amendment is a Rule 132 Declaration by Dr. William O. Dawson and a Rule 132 Declaration by Dr. Laurence K. Grill. Also submitted herewith is Goldbach, New Aspects Of Positive-Strand RNA Viruses (M. Brinton & F. Heinz, ed.), American Society for Microbiology, Washington, D.C. pp.3-11 (1990) [EXHIBIT 1], R. E. F. Matthews, Plant Virology, 3rd Edition, Academic Press, Inc., San Diego p.180 (1991) [EXHIBIT 2] and Kumagai, et al., Proc. Natl. Acad. Sci. (1993) 90:427-430 [EXHIBIT 3]. Consideration of the present application on the merits is respectfully requested in

view of the amendments to the Specification, new claims 45-82, the declarations submitted herewith and the following remarks.

I. Amendment To The Specification Claim Priority To Continuation-In-Part Application 07/739,143

Applicants have amended the Specification to cross-reference and claim priority to copending Application Serial No. 07/739,143 filed August 1, 1991. The present application is a continuation-in-part application of the '143 application.

II. Entry Of New Claims 45-82 Is Proper Since No New Matter Is Being Introduced

Applicants respectfully request entry of new claims 45-82 since these claims do not introduce new matter. Independent claims 45 and 67 require that the plant virus be a plus sense, single stranded RNA plant virus. The Specification teaches that "the genome of most monopartite plant RNA viruses is a single-stranded molecule of (+)-sense.... An example of this type of virus is TMV." Specification, page 3, lines 17-21. With regard to the requirement that the plant virus naturally have a subgenomic promoter, the Specification teaches that "recombinant plant viral nucleic acids according to the present invention comprise a native plant viral subgenomic promoter." Specification, page 6, lines 12-13.

Independent claims 45 and 67 require that the nucleic acid comprise a first subgenomic promoter that regulates transcription of a coat protein and a second subgenomic promoter that regulates the transcription of a second nucleic acid sequence. The Specification teaches that the recombinant plant viral nucleic acids according to the present invention comprise "a native plant viral subgenomic promoter, at least one non-native plant viral subgenomic promoter, a plant viral

coat protein coding sequence and, optionally, at least one non-native nucleic acid sequence." Specification, page 6, lines 12-16. The Specification teaches and it would be understood in the art that the "inserted ... subgenomic promoters are capable of transcribing or expressing adjacent genes in a plant host." Specification, page 7, lines 21-25.

Independent claims 45 and 67 also require that the first and second viral subgenomic promoters possess different nucleic acid sequences enabling the recombinant plant viral nucleic acid to systemically transcribe the second nucleic acid in the host plant.

The Specification teaches that the viral constructions of the present invention "are stable for the maintenance and transcription or expression of non-native (foreign) nucleic acid sequences in the host plant." Specification, page 6, lines 6-9.

The Specification teaches that instability is associated with homologous recombination of repeated sequences. Specifically, the Specification teaches that

> it was hypothesized that with the previously reported constructs, foreign inserts were deleted due to recombination between repeated subgenomic promoter sequences.

Specification, page 58, lines 5-8. Additionally, the Specification teaches that "since the deletion occurred between repeated sequences, it is possible that this occurred by homologous recombination as described for other plus-sense RNA viruses." Specification, page 49, lines 24-28.

In order to combat the problem in the art regarding unstable vectors, the present invention requires that the non-native subgenomic promoters be "incapable of recombination with each other and with native subgenomic promoters." Specification, page 7,

lines 24-25. The Specification teaches that this result is achieved by reducing the number of repeated sequences by using heterologous subgenomic promoters. Specification, page 58, lines 8-10. Therefore, the Specification supports the requirement that the "first and second viral subgenomic promoters possess different nucleic acid sequences."

"Different" is defined functionally in claim 46 as a difference sufficient to enable "the recombinant plant viral nucleic acid to systemically transcribe the second nucleic acid in the host plant." This definition of "different" is supported by the Specification which teaches that homologous recombination of repeated sequences prevents systemic transcription. Specification, page 49, lines 20-28. Therefore, by employing subgenomic promoters whose sequences are sufficiently "different" so as to reduce the frequency of homologous recombination of repeated sequences, systemic transcription is enabled. Specification, page 11, lines 3-13.

The degree to which the nucleic acid sequences of the first and second subgenomic promoters need to differ to prevent recombination will vary between vectors constructs. The present invention teaches, by way of example, that subgenomic promoters that are not native to the same virus are sufficiently different for the purpose of the present invention. Applicants maintain that whether two subgenomic promoters have sufficiently different nucleic acid sequences to enable systemic expression of the second nucleic acid can be determined by one of ordinary skill without undue experimentation by testing for systemic expression.

New claims 46-51, 57-62 and claims 73-78 contain claim limitations regarding whether the first subgenomic promoter, the second subgenomic promoter or the coat protein is native to the recombinant viral

nucleic acid. These claim limitations are supported by the Specification at page 6, line 4 to page 7, line 35.

New claim 52 specifies the incorporation of a third subgenomic promoter and a third nucleic acid sequence. This claim limitation is disclosed in the Specification at page 12, lines 3-16.

New claims 53 and 79 are supported by the fact that the present invention encompasses recombinant plant viral nucleic acids derived from a (+) sense, single stranded RNA plant virus possessing a native subgenomic promoter. The viral species listed in new claims 53 and 79 all satisfy this claim limitations and thus inherently fall within the scope of the present invention. See Goldbach, New Aspects Of Positive-Strand RNA Viruses (M. Brinton & F. Heinz, ed.), American Society for Microbiology, Washington, D.C. pp.3-11 (1990) [EXHIBIT 1]. Therefore, claims 48 and 74 do not introduce new matter.

New claims 54, 55 and 82 require that the recombinant plant viral nucleic acid be derived from a tobamovirus and, more specifically, TMV. The Specification clearly teaches that the tobamovirus and TMV are within the intended scope of the present invention.

Finally, claims 68-72 relate to expression and isolation of the second nucleic acid sequence, the protein encoded for by the second nucleic acid sequence and a secondary metabolite. Support for new claims 68-72 is found in the Specification at page 8, lines 10-15 and page 24, lines 31-32. Therefore new claims 68-72 are supported by the Specification and do not introduce new matter.

Finally, new claims 81-82 are supported by original claims 41-42 and therefore do not introduce new matter.

Applicants have demonstrated that new claims 45-82 are supported by the Specification. Applicants therefore maintain that entry of new claims 45-82 is proper since no new matter is being introduced.

III. Response To Telephone Restriction Requirement

On November 19, 1993, Examiner issued a telephone restriction requirement alleging that the present application claims three distinct inventions. Specifically, Examiner has divided the application into claims 1-42 which are drawn to recombinant plant viral nucleic acid (Group I), claim 43 which is drawn to pTB2 (Group II) and claim 44 which is drawn to pTBU5 (Group III).

Pursuant to 37 C.F.R. § 1.142, Applicants hereby provisionally elect Group I (new claims 1-42) but reserve the right to traverse Examiner's restriction requirement pursuant to 37 C.F.R. § 1.144. These claims are presently cancelled by the present amendment and are replaced by new claims 45-82. Claims 43 and 44 may be withdrawn from further consideration by the Examiner under 37 CFR 1.142(b), as being drawn to a non-elected invention.

Applicants traverse Examiner's restriction requirement with regard to Inventions I, II and III on the grounds that these three sets of claims do not represent separate and distinct inventions as is required to support Examiner's restriction requirement. Claims 43 and 44 claim the TB2 vector and the TBU5 vector, two vectors "prepared in accordance with the present invention." Specification, page 8, lines 16-17. Since these two vectors are further embodiments of Invention I, Applicants maintain that claims 43 and 44 do not represent separate and distinct inventions that are properly subject to restriction. Since claims 43 and 44 are actual embodiments of Invention I, it is

clear that no additional search would be required. Applicants therefore respectfully request Examiner to withdraw the present restriction requirement.

Applicants also reserve the right pursuant to 35 U.S.C. § 121 to file one or more divisional applications directed to the cancelled claims during the pendency of the present application.

VI. Brief Description Of The Present Invention

The present invention relates to a recombinant plant viral nucleic acid possessing enhanced stability within a host plant, thereby enabling the sustained systemic transcription of a nucleotide sequence within the host. Enhanced stability within the host plant has been accomplished by the use of a dual subgenomic promoter system which is believed to reduce the frequency of recombination leading to the regeneration of the wild type virus.

Specifically, the recombinant plant viral nucleic acids of the present invention comprise a first viral subgenomic promoter, a nucleic acid sequence that codes for a plant viral coat protein whose transcription is regulated by the first plant viral subgenomic promoter, a second plant viral subgenomic promoter and a second nucleic acid sequence whose transcription is regulated by the second plant viral subgenomic promoter. The requirement that the recombinant plant viral nucleic acid comprise a second nucleic acid that is not naturally associated with the plus sense single stranded RNA plant virus from which the nucleic acid is derived distinguishes the recombinant plant viral nucleic acid from nature.

Subgenomic promoters are defined in the Declaration by Dr. Laurence K Grill, which is submitted herewith. Dr. Grill notes that

> a number of single-stranded RNA viruses have smaller, subgenomic mRNAs that are synthesized in the host's cells after infection. The transcription of these subgenomic RNAs depend on sequences that are upstream (5') and are referred to as internal or subgenomic promoters.

A good description of subgenomic promoters is presented in R. E. F. Matthews, Plant Virology, 3rd Edition, Academic Press, Inc., San Diego p.180 (1991) which is attached hereto as EXHIBIT 2.

The recombinant plant viral nucleic acids of the present invention systemically express the second nucleic acid sequence within the infected host. Systemic expression is enabled by the difference in the nucleic acid sequences between the first and second subgenomic promoters which serves to inhibit recombination of the subgenomic promoters with each other and other parts of the viral genome to yield the wild type virus. As a result, the recombinant plant viral nucleic acids of the present invention are sufficiently stable within the host plant to enable the sustained systemic transcription of the second nucleic acid sequence. Prior art vectors used the same subgenomic promoter (Ahlquist, et al.) and were not able to achieve systemic transcription of a foreign nucleic acid sequence. By contrast, Applicants have accomplished the highest accumulation of a foreign protein ever reported in any genetically engineered plant using a vector designed according to the present invention . See Kumagai, et al., Proc. Natl. Acad. Sci. (1993) 90:427-430 [EXHIBIT 3].

V. Inventorship Of The Present Invention

In U.S. Serial No. 07/739,143, the Examiner questioned the inventorship of the application. In response, Applicants submitted a Rule 132 Declaration by Dr. William O. Dawson attesting to the inventorship of the application. The present application is a continuation-in-part of the '143 application. The statements made in the Declaration by Dr. Dawson regarding the inventorship of the '143 application apply equally to the inventorship of the present application.

The Declaration by Dr. Dawson explains that neither Christopher M. Kearney nor Mark E. Hilf contributed to the conception or reduction to practice of the present invention and therefore are not coinventors of the above-referenced application.

The Declaration by Dr. Dawson further states that each of the named coinventors, specifically, Jon Donson, George L. Grantham, Thomas H. Turpen, Ann M. Turpen, Stephen J. Garger, Laurence K. Grill and Dr. Dawson,

> contributed to the conception and reduction to practice of at least one of the pending claims and therefore should be considered a co-inventor of the above-referenced application.

In view of the declaration by Dr. Dawson, Applicants maintain that the Examiner has no reason to doubt the inventorship of the present application.

VI. Enablement Of Claims 45-82

The Examiner has no reasonable basis for doubting the enablement of the present invention as it is presently claimed. In order to properly challenge enablement, the Examiner must first set forth an

evidentiary basis for questioning the adequacy of a disclosure. Gould v Mossinghoff, 229 U.S.P.Q. 1, 10 (1985). This is because a disclosure is presumptively enabled. Gould, 229 U.S.P.Q. at 10. In order to demonstrate lack of enablement, the Patent and Trademark Office has the initial burden of providing specific reasons as to why the Specification and record as a whole is not enabling such that undue experimentation is required to practice the invention. In re Armbruster, 512 F.2d 676 at 677 (C.C.P.A. 1975).

The present invention is grounded upon the realization that it is possible to stabilize a recombinant viral nucleic acid by using a dual subgenomic promoter system where the nucleic acid sequences for the subgenomic promoters are sufficiently different so as to reduce the frequency of recombination leading to the regeneration of the wild type virus.

There is no reason for the Examiner to doubt that the dual promoter concept will not be equally applicable to all plus sense, single stranded viruses that naturally possess a subgenomic promoter. As stated in the Declaration by Dr. Laurence K. Grill, "any recombinant viral nucleic acid derived from a plus sense, single stranded RNA virus that naturally has a subgenomic promoter" may be employed in the present invention. Plus sense, single stranded RNA viruses that naturally possess a subgenomic promoter include tobamoviruses, bromoviruses, tobraviruses, furoviruses, cucumoviruses, hordeiviruses, potexviruses, tymoviruses, luteoviruses, carmoviruses, tombusviruses, sobemoviruses and alphaviruses such as the sinbis virus. Each virus is capable of infecting a different group of organisms.

Subgenomic promoters from a variety of plus-sense RNA viruses have been employed in the present

invention. Table 1 of the Grill Declaration describes a series of functional vectors that have been prepared according to the present invention. Subgenomic promoters used in the vectors described in Table 1 include tobacco mosaic virus (TMV) and odontoglossum ringspot virus (ORSV).

The functional vectors depicted in Table 1 demonstrate that neither the subgenomic promoter that regulates transcription of the coat protein nor the subgenomic promoter that regulates transcription of the second nucleic acid sequence needs to be naturally associated with the plant virus from which the recombinant viral nucleic acid is derived. As Dr. Grill notes, "given that transcription of the coat protein and the second nucleic acid sequence are not interdependent, there is no requirement that either the subgenomic promoter for the coat protein sequence or the second nucleic acid sequence be native to the vector."

In addition, the Declaration of Dr. Laurence K. Grill submitted herewith clearly supports Applicants' position that the particular coat protein, subgenomic promoter and structural orientation of the vector are not critical to the production of a functional vector.

In view of the data presented in the Specification and the Rule 132 Declaration by Dr. Laurence K. Grill, Applicants maintain that the Examiner lacks a reasonable basis for rejecting new claims 45-82 for lack of enablement.

VII. Novelty Of Present Invention Over Ahlquist, et al.

In order for a claim to be anticipated by a reference, that reference must disclose each and every claim limitation. New claims 45-82 are not anticipated by Ahlquist, et al. because Ahlquist, et al. neither teaches nor suggests that the subgenomic promoter for

the coat protein must not be able to recombine with the subgenomic promoter for the heterologous nucleic acid sequence. Rather, Ahlquist, et al. teaches that the foreign gene may be expressed either as a fusion protein or by using the same subgenomic promoter as is used to express the coat protein. See Ahlquist, et al., Col. 9, lines 48-58.

The comparative examples presented in the Specification clearly teach that vectors where the foreign gene is expressed as a fusion protein (Comparative Example 1, Specification, page 42) or where the same subgenomic promoter is used (Comparative Example 2, Specification, page 50) are not able to systemically infect the host due to recombination. By contrast, the Specification demonstrates that a vector which employs two different subgenomic promoters systemically infects the host plant without loss of expression of the foreign gene. Example 1, Specification, page 53. Using a vector designed according to the present invention, Applicants have been able to accomplish the highest accumulation of a foreign protein ever reported in any genetically engineered plant. See Kumagai, et al., Proc. Natl. Acad. Sci. (1993) 90:427-430 [EXHIBIT 3]. Thus, the requirement that two different subgenomic promoters be used is a functionally significant difference between the present invention and Ahlquist, et al.

Since Ahlquist, et al. does not teach at least one of the limitations of claims 45-82, Ahlquist, et al. cannot be said to anticipate the present invention.

VIII. Nonobviousness Of New Claims 45-82 Over Takamatsu, et al. And Gallie, et al. Taken With Ahlquist, et al.

The present invention requires the first and second viral subgenomic promoters to possess different nucleic acid sequences in order to prevent

recombination leading to the wild-type virus, thereby enabling the recombinant plant viral nucleic acid to systemically transcribe the second nucleic acid in the host plant.

The present invention was developed in order to overcome existing problems in the art with recombination. For example, Donson, et al. teaches that

> vector constructs have also been constructed with an additional viral subgenomic promoter to express a foreign gene. However, on infection of plants, these vectors had the added sequences deleted and failed to be transported systematically. This was hypothesized to be from recombination between the two repeated subgenomic promoter sequences within the viral constructs.

Donson, et al., page 7204, column 2.

As Applicants discussed with regard to the novelty of the present invention, Ahlquist, et al. neither teaches nor suggests using two different subgenomic promoters for the coat protein and heterologous protein sequences in order to prevent recombination. Further, neither Takamatsu, et al. nor Gallie, et al. teach or suggest Applicants' use of two different subgenomic promoters to solve the existing problem in the art regarding unstable vectors due to recombination. Since the above noted combination of references fails to teach the most novel and crucial aspect of the present invention, these references do not render new claims 45-82 unpatentable for obviousness.

CONCLUSION

Applicants respectfully request entry of the present new claims. Applicants submit that the present amendments to the claims and the Specification are proper because no new matter is being introduced.

In addition, Applicants earnestly believe that they are entitled to a letters patent in view of the present amendments and respectfully solicit Examiner to expedite prosecution of this patent application to issuance.

Respectfully submitted,
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